DDTによる昆虫神経陰性後電位の増大 : 殺虫剤の作用機構に関する研究 第13報

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by organic solvent. A certain quantity of the extract was decomposed quantitatively by 1N-methanolic KOH, and the amount of KCl was determined by Volhard method. Thus, the portion of BHC not decomposed was determined and from these data the heat decomposition rate calculated.

Determination of BHC existing in the two components system of BHC-trichlorobenzene can be also accomplished by this method. The heat decomposition rate calculated from the quantity of BHC contained in the aerosol of BHC smoke fumigant agreed within a exeperimental error with the one calculated through quantitative determination of inorganic chlorine generated by the heat decomposition.

Increase in the Negative After-potential of Insect Nerve by DDT. Studies on the Mechanism of Action of Insecticides. XIII. Teruo YAMASAKI and Toshio NARAHASHI*
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51. DDT による昆虫神経陰性後電位の増大 殺虫剤の作用機構に関する研究 第13報 山崎輝男・楢橋敏夫**（東京大学農学部害虫学研究室）32. 5. 30 受理

DDT の作用機構を究明するためには，その作用点である神経の DDT による機能変化を種々の観点から詳細に調べることが，当面する重要な課題である．DDT が昆虫神経の陰性後電位を増大，延長させることは，筆者らの初期の研究において明らかにされているが，今回はこの点につきさらに詳細な実験を行つた．陰性後電位は DDT 中毒の潜伏期より痙攣期に至るまでの中毒初期において著るしく増大，延長し，また低温において症状を呈している虫を高温に移して回復させたものでも，増大した陰性後電位は変化しなかつた．このような後電位の変化とともに，神経の回復過程も DDT によつて影響され，著るしい過常期を示すようになつた．以上の実験結果を既存のデータと照らし合わせて考察した．

A hypothesis concerning the mode of toxic action of DDT upon insect nerve was proposed in our previous paper[32]. The resting potentials of insect nerves under the influence of DDT were compared with those of the nerves treated with nitrogen, metabolic inhibitors, and potassium ions. It was suggested that DDT has no effect on the "resting metabolism" of nerve but affects the ionic permeability either by a disturbance of the "active metabolism" of the nerve or by a direct physico-chemical action on the nerve membrane. In order to demonstrate this hypothesis, it is necessary to perform a more detailed analysis of the changes in nerve function caused by DDT. In one of our early studies in which the effects of insecticides on the spontaneous activity of the central nerve cord were examined, it was found that DDT markedly augmented and prolonged the negative after-potential. Evidence was presented indicating that the after-potentials are closely related to the excitability. The positive after-potential is related to the depression of excitability and the negative after-potential to augmentation, the after-potentials are much more sensitive to changes in environmental factors than the spike-potentials, and they may actually be some manifestations of the recovery process. Taking such views into account it is also necessary to perform more detailed analyses of the DDT induced negative after-potential in order to understand the ionic or metabolic events occurring in a nervous system under the influence of DDT. This paper will present some important data concerning the mode of action of DDT upon nerve.

Materials and Methods

Insects: Both adults and large nymphs of the American cockroach, *Periplaneta americana* L., reared in the laboratory, were used throughout the experiments. Since no differences concerning the effect of DDT on the action potential were found between adults and large nymphs, the results

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obtained will be discussed together.

Nerve preparations : The cockroach cercal nerve, which includes mainly sensory nerve fibres originating in the cercus, is connected synaptically to the giant axons in the sixth abdominal ganglion, and these giant axons run through the abdominal nerve cord, and then in turn either have synaptic contacts with the motoneurones in the meta-, meso-, or pro-thoracic ganglion, or run directly to the brain. The crural nerve includes large motoneurones which originate in the thoracic ganglion and innervate the muscles of the leg. Thus a so-called "evation reflex", starting at the cercus, passing through the cercal nerve, the giant axons and the motoneurones, and reaching the muscles of the leg, is formed[17]. This reflex system offered us a good opportunity to record the action potentials of single nerve fibres *in situ*, because a tactile stimulation applied to the cercus evokes a series of reflex discharges in which the action potentials of each motoneurone can easily be observed separately. The action potentials of the crural nerve were thus observed *in situ* in the present study. As the giant axons in the nerve cord are very large in diameter compared with other axons in the nerve cord, resting and action membrane potentials, recorded at any point of the nerve cord by means of external electrodes are largely due to the membrane potentials originating in the giant axons[32]. The action potentials of the isolated nerve cord were also observed in this study.

Treatment with DDT : The cockroach was topically treated with DDT, or the nerve preparation excised from the untreated cockroach was treated with DDT suspended Ringer solution. In the former case, pure *p,p'*-DDT acetone solution was applied to the dorsal surface of the abdomen, and then the poisoned cockroaches were dissected after various time intervals. The poisoning symptoms of DDT can be divided into five stages : latent period, period of locomotor instability, convulsive period, paralytic period, and death[28]. Since the last two stages are not caused by a direct primary action of DDT but are the secondary effects of DDT poisoning[29], only the roaches showing any of the first three stages of poisoning symptoms were examined. It has been demonstrated that the insecticidal action of DDT is stronger at low temperature than at high and that the poisoning symptoms of DDT are reversible when an insect poisoned at low temperature is transferred to a higher one. These phenomena can clearly be explained by the higher susceptibility of the insect nerve to DDT at low temperature; the threshold concentration of DDT for initiation of repetitive excitation at a low temperature is lower that at a higher temperature[30]. The action potentials of the nerves of roaches which had recovered from DDT poisoning after being transferred from a low temperature to a higher one were also measured.

DDT suspended Ringer was prepared by an injection of *p,p'*-DDT ethanol solution into Ringer solution. Ethanol alone at the concentration used had no effect on the action potentials.

The Ringer solution used was the same as that described in our preceding paper[32].

Stimulations and recordings : The motoneurone discharges in the crural nerve were elicited by a tactile stimulation of the cercus. The action potentials of the crural nerve of the meta-leg were recorded *in situ* by means of one pair of fine silver electrodes, one of which was in contact with an uninjured region of the crural nerve, while the other was in contact with a cut and crushed region of the crural nerve at a distal part of the coxa. Since a detailed analysis of the action potentials was not intended in this experiment with the crural nerve, it was unnecessary to use non-polarizable electrodes.

The recording of the action potentials of the nerve cord was made by means of one pair of fine silver-silver chloride non-polarizable electrodes or one pair of Zn-$ZnSO_4$ Ringer-gelatin type non-polarizable electrodes, one of which was in contact with a central uninjured region of the nerve cord, while the other was in contact with the sixth abdominal ganglion crushed with forceps. A single action potential was elicited by a single electrical stimulus which was delivered to the anterior peripheral region of the nerve cord through one pair of fine silver electrodes. The electrical stimuli were either condenser discharges

or discharges from an elctronic stimulator intervening inductorium.

In the experiment for determining the recovery curve, two successive condenser discharges with various time intervals were applied to the nerve cord through one pair of stimulating electrodes, while the action potentials evoked were recorded as described above. A conditioning shock was either a maximum or a supramaximum stimulus, while a maximum or a just supra-threshold stimulus was employed as a testing shock.

The action potentials recorded from the crural nerve were amplified by a RC-coupled amplifier with a time constant of 0.25 second, and those recorded from the nerve cord were amplified by a RC-coupled amplifier with a time constant of 1.0 second or a DC amplifier. The amplified action potentials were observed by means of a cathode ray oscilloscope.

Results

Action Potential of the Crural Nerve

The action potentials recorded from the crural nerve of the normal cockroach usually showed a slight overshoot. In some cases no overshoot was observed, however, the negative after-potential was too small to be detectible in any case(Fig. 1).

The negative after-potential was augmented and

Table 1. The duration of the action potential in the crural nerve of the cockroach poisoned with DDT.

Time and temperature after treatment	Temperature (°C) at observation	Stage* of symptom	Mean duration of action potential (msec.)	No. of insects examined	No. of action potentials measured
Control	16.5		1.73	8	91
Control	29		1.45	2	9
1 day at 14~17°C	17	I	3.64	4	90
	17	II	5.11	1	16
2 days at 14~17°C	17.5	II	3.16	2	45
	17	III	4.93	1	34
1 day at 14~17°C and 1 day at 29°C	29	I′	3.74	2	87
	29	II	6.24	2	46
1 day at 14~16°C	17	I	3.88	3	39
	17	II	6.73	5	38
	17	III	2.18	1	25
1 day at 14~16°C and 1 day at 29°C	29	I′	5.54	4	69
1 day at 14~16°C	15	II	2.63	5	71
	15	III	10.28	3	12
1 day at 12~14°C	14	II	2.17	4	52
	14	III	2.97	2	15
2 days at 12~14°C	14	II	2.56	4	46
	14	III	2.15	2	26
1 day at 12~14°C and 5~6 hours at 29°C	29	I′	2.28	6	66
4 hours at 14~16°C	15	I	1.84	4	51
1.5~2 hours at 14~16°C	15	II	2.14	2	58
3 hours at 14~16°C and 2 hours at 29°C	15	II	1.94	1	18
3 hours at 14~16°C and 3 hours at 29°C	15	III	6.41	2	37
8 hours at 14~16°C	15	II	2.08	1	13

* Stage of symptom

I : latent period or no symptom

II : ataxia and severe convulsion

III : complete ataxia and weak convulsion

I′ : complete recovery upon rising the temperature

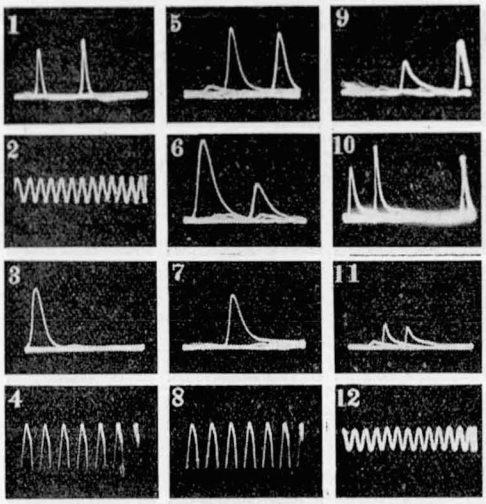

Fig. 1. Action potentials of the crural nerve of the cockroach evoked by a tactile stimulation of the cercus. Each action potential is not a compound but a single action potential of each motoneurone.

1, untreated, slight positive overshoots, 16°; 2, 700c. p. s. for record 1; 3, untreated, no overshoot; 4, 1000c. p. s. for record 3; 5 and 6, treated with DDT, latent period, slightly prolonged negative after-potentials, 17°; 7, ataxia and severe convulsion, prolonged negative after-potential, 17°; 8, 1000c. p. s. for records 5 to 7; 9, weak convulsion, markedly augmented negative after-potential, 17°; 10, recovered upon raising the temperature, the prolonged negative after-potentials are not restored, 29°; 11, ataxia and severe convulsion, markedly augmented and prolonged negative after-potentials, 29°; 12, 700c. p. s. for records 9 to 11.

prolonged markedly in the DDT poisoned roach at low temperature. Measurements of the duration of the action potential revealed that in the poisoned roach it was usually longer than 2 milliseconds, often over 10 milliseconds, while that of the control or the unpoisoned roach was 1.73 millisecond at 16.5° (Fig. 1). The results are summarized in Table 1. The absolute values of the duration of the action potentials were somewhat different in each series, but it could be clearly observed that the duration of the action potential was prolonged in all stages of the poisoning symptoms examined, even in the latent period. But no tendency to increase in duration as poisoning progressed was observed. Furthermore, the prolonged action potential was maintained in the roach in which recovery had occurred by increasing the temperature to 29° (Fig. 1, Table 1). The action potential of the normal untreated nerve had a mean duration of 1.45 millisecond at 29°. The same events occurred when the action potential of the crural nerve was recorded from one side of a poisoned roach at a low temperature, and then was recorded again from the other side of the same roach after the disappearance of the symptoms on raising the temperature (Table 2). These results indicate that although the symptoms of DDT poisoning disappears upon raising the temperature, the mechanism of the prolongation of the action potential is not affected by the same procedure.

Table 2. The duration of the action potential in the crural nerve of the cockroach poisoned with DDT. The action potential was recorded at 16° in the left leg of the cockroach showing the symptom of ataxia and severe convulsion 1 day after treatment. The cockroach was then kept at a higher temperature, 29°, for 1 day, and the action potential was again recorded at 29° in the right leg after the disappearance of the poisoning symptoms.

No.	Mean duration of action potential (msec.) (No. of action potentials measured)	
	16°C	29°C
1	2.41 (11)	6.08 (7)
2	10.29 (5)	3.12 (5)
3	3.28 (19)	5.63 (12)
4	1.89 (14)	2.45 (10)
5	4.46 (10)	3.13 (9)
6	4.40 (1)	7.78 (9)
7	1.90 (3)	3.87 (5)
Mean	4.09	4.58

Action Potential of the Central Nerve Cord

After-potential : As mentioned before, the action potentials recorded in the central nerve cord of the roach are largely due to the action potentials of the giant axons. This compound action potential often showed a slight positive overshoot following a negative spike. In the roach poisoned with DDT at a low temperature the after-negativity of the action potential was augmented and markedly prolonged, often lasting over 30 milliseconds (Fig. 2).

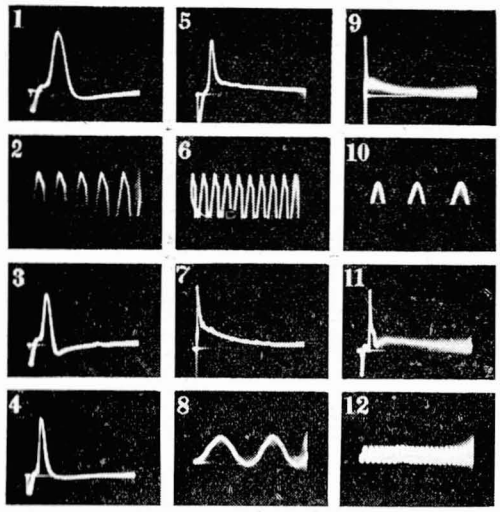

Fig. 2. Action potentials of the central nerve cord of the cockroach evoked by a single stimulus. 1, untreated, slight positive overshoot, 16°; 2, 1000c. p. s. for record 1; 3, untreated, slight positive overshoot, 16°; 4, untreated, no overshoot, 18°; 5, treated with DDT, weak convulsion, increased negative after-potential, 16.5°; 6, 1000c. p. s. for records 3 to 5; 7, weak convulsion, markedly increased negative after-potential, 16.5°; 8, 50c. p. s. for record 7; 9, the same as record 7, slower sweep; 10, 50c. p. s. for record 9; ataxia and severe convulsion, separation of the spike-potential and the negative after-potential, 16.5°; 12, 700c. p. s. for record 11.

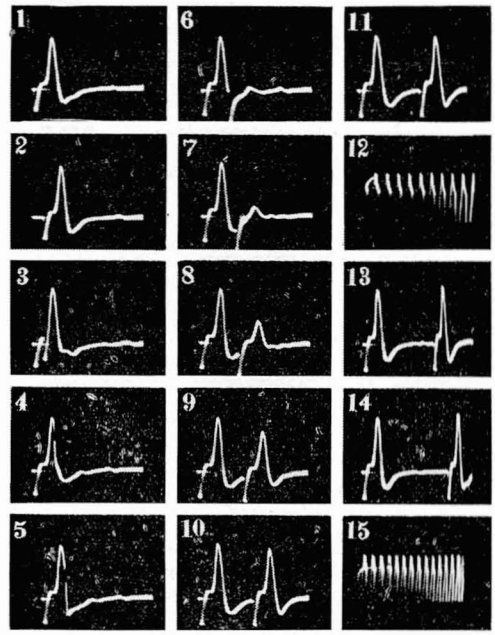

Fig. 3. A series of oscillograms of the action potentials evoked by two successive maximum stimuli at various time intervals which shows the absolute and the relative refractory period. The central nerve cord of the untreated cockroach, 16°. 1, a conditioning stimulus alone; 2, a testing stimulus alone; 3 to 11, a conditioning and a testing stimulus; 12, 1000c. p. s. for records 1 to 11; 13 and 14, the same as records 3 to 11; 15, 1000c. p. s. for records 13 and 14.

Recovery process : In the normal or untreated nerve, an action potential is followed by an absolute refractory period and then by a relative refractory period. When two successive maximum or supramaximum stimuli were delivered through the same pair of electrodes, the second stimulus was ineffective at intervals of less than 1.6～2.1 msec., which indicated the absolute refractory period. When the intervals between a conditioning and a testing shock were increased by longer than the absolute refractory period, the testing shock generated the action potential, the height of which grew as the shock interval increased progressively, and eventually attained the level of the action potential evoked by the testing shock alone.

An example of a series of such oscillograms is shown in Fig. 3, and also one of a recovery curve is illustrated by Fig. 4.

When a submaximum stimulus is applied as a testing shock after a supramaximum conditioning stimulus, the supernormal phase, if it were present, would be demonstrated. In the case of the nerve of the normal cockroach, however, the supernormal phase was not observed, as is shown by the series of oscillograms and the recovery curve in Fig. 5 and Fig. 6 respectively.

The effect of DDT on the recovery process was clearly demonstrated. It can easily be observed in Fig. 7 that the nerve excised from a DDT poisoned cockroach in which a submaximum testing stimulus is applied to the nerve with various time intervals after a supramaximum conditioning stimulus shows a marked supernormal phase. A recovery curve in the poisoned nerve is plotted in Fig. 8 together with a tracing of the negative after-potential induced by the DDT poisoning. It is evident from such experiments

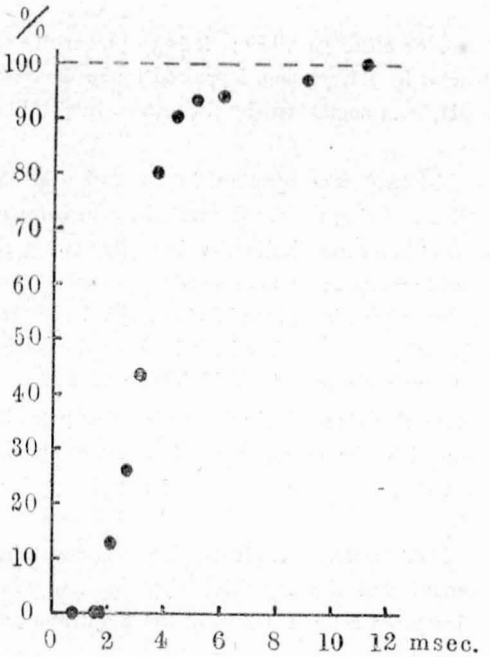

Fig. 4. Recovery process of the giant nerve fibres showing the absolute and the relative refractory period, which is drawn from the same experiment illustrated in Fig. 3. Percentages of the spike height of the testing response to that of the conditioning one is taken as ordinates, and the intervals between the conditioning and the testing shock as abscissae.

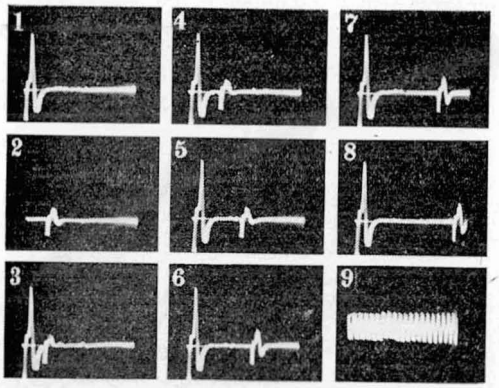

Fig. 5. A series of oscillograms of the action potentials evoked by two successive stimuli at various time intervals, which shows the absence of the supernormal phase. The central nerve cord of the untreated cockroach, 16°. 1, a maximum conditioning stimulus alone; 2, a testing stimulus of just above threshold alone; 3 to 8, a conditioning and a testing stimulus; 9, 700c. p. s. for all records.

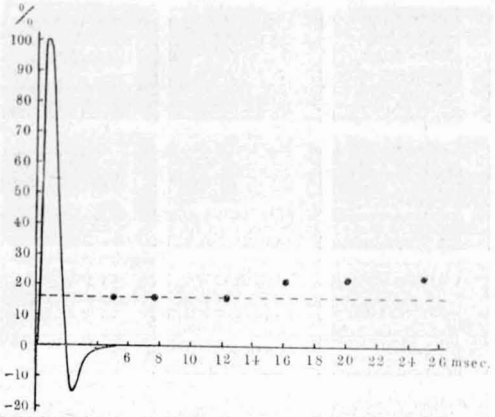

Fig. 6. Recovery process of the giant nerve fibres showing the absence of the supernormal phase, which is drawn from the same experiment illustrated in Fig. 5, superimposing a normal action potential upon it. Percentages of the spike height of the testing response to that of the conditioning one is taken as ordinates, and the intervals between the conditioning and the testing shock as abscissae for drawing the recovery process. The broken line shows the level of response by a testing shock alone. For drawing the action potential, the spike height is taken as ordinates and the time after the onset of the action potential as abscissae.

that an increase in the negative after-potential induced by DDT is accompanied by an increase in the supernormal phase.

Discussion

The nature of the after-potentials have widely been investigated in both the myelinated and the non-myelinated nerve fibres, and the data accumulated have indicated some important features as follows :

A parallelism between the after-potential and the recovery phase can be made to some extent ; the duration of the negative after-potential is matched by the duration of the supernormal phase, and that of the positive after-potential is matched by that of the subnormal phase[3,5,8,11,13]. The negative after-potential was shown to be very sensitive to changes in various environmental factors; it is depressed earlier than the spike-potential when the nerve is placed in an environment lacking oxygen or is exposed to carbon monoxide[1,6,11,15,21] ; it is easily depressed by

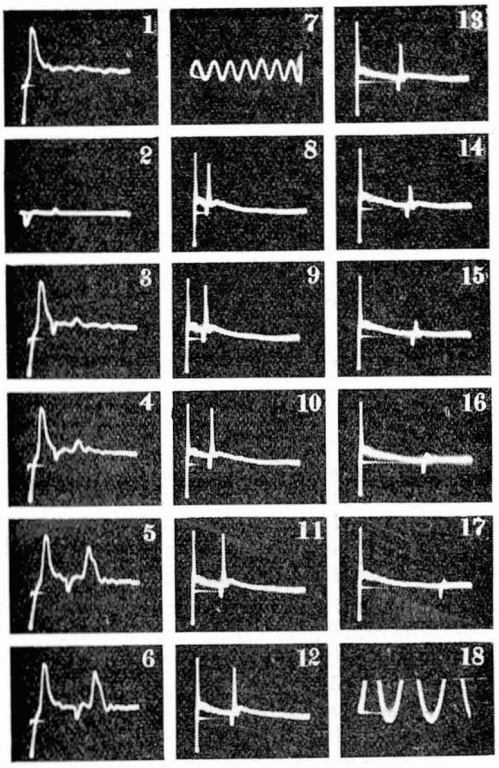

Fig. 7 A series of oscillograms of the action potentials evoked by two successive stimuli at various time intervals, which shows both the increase in the negative after-potential and the presence of the supernormal phase. The central nerve cord of the DDT-treated cockroach showing ataxia and severe convulsion, 16.5°, 1, a maximum conditioning stimulus alone; 2, a testing stimulus of just above threshold alone; 3 to 6, conditioning and a testing stimulus; 7, 700c. p. s. for reords 1 to 6; 8 to 17, the same as records 3 to 6; 18, 50 c. p. s. for records 8 to 17.

certain narcotics, i. e., procaine, cocaine, urethane, or ether gas[8,11,22]; it is very sensitive to changes in pH, being depressed by an increase in pH, and augmented by a decrease in pH[4,13]; it is also very sensitive to changes in potassium and calcium concentration in the surrounding medium, being usually depressed by an increase in potassium concentration, and usually augmented by an increase in calcium concentration[10,14,22-24]. The negative after-potentials in any kind of nerve are usually augmented and markedly prolonged by treatment with veratrine[2,5,8,9,11,16,19-25].

Since there is no doubt that each action potential observed in the crural nerve is not a compound but a single one, an augmentation and a prolongation of the negative after-potential observed in the DDT treated nerve is not due to a smoothing summation of the repetitive after-discharges but is due to an actual increase in the negative after-potential. However, a detailed analysis of the shape of the action potential cannot be made in the experiments with the crural nerve, because the shape of the action potential shows some variations even in the control experiments, and also because an action potential recorded with external electrodes undoubtedly does not show its actual shape and height but only relative ones. It was actually observed that the duration of the action potential was somewhat increased when the nerve was slightly dessicated.

The increase in the negative after-potential of

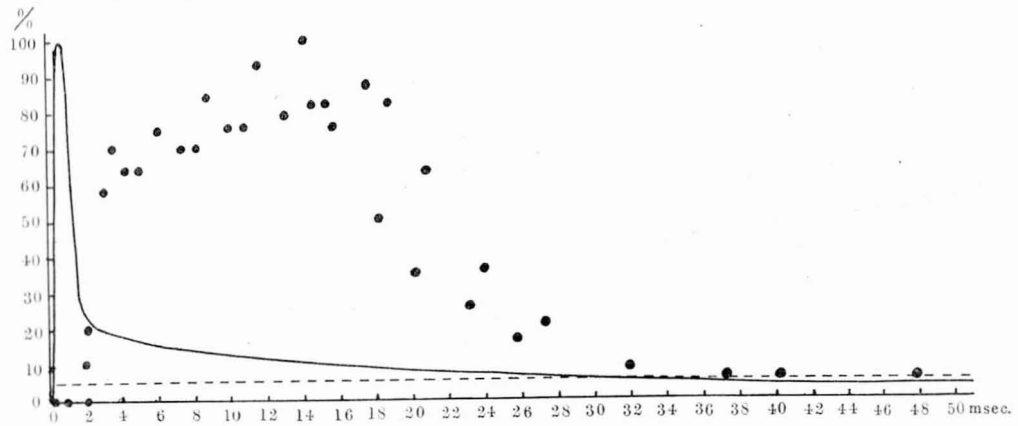

Fig. 8. Recovery process of the giant nerve fibres of the DDT-treated cockroach developing ataxia and severe convulsion which shows a marked supernormal phase. This is drawn from the same experiment illustrated in Fig. 7, superimposing an action potential with prolonged negative after-potential caused by the DDT treatment upon it. Both abscissa and ordinate are the same as those of Fig. 6.

the insect nerve by treatment with DDT has never been reported before. Only in the crab nerve was such a change in the negative after-potential observed after treatment with DDT[23], but in the squid giant axon it was not found after the same treatment[22].

The supernormal phase which cannot be observed in the untreated nerve is markedly increased by treatment with DDT and shows good agreement with the course of the increased negative after-potential. Such parallelism between the after-potential and the recovery phase is also found in the nerve of other animals as described.

In the light of such considerations as these, the augmentation and prolongation of the negative after-potential by DDT poisoning must afford a very important key point in the solution of the mode of action of DDT on nerve. On the other hand, it has already been demonstrated that DDT affects several other characters of the nerve function; it augments the repetitive excitability in both the soma and the axon[7,26,27,31]; the action of DDT on the repetitive excitability is markedly enhanced by the lowering of the calcium concentration in the surrounding medium[7]; it also induces repetitive discharges in the sensory nerve cells[12,18,29]; it increases the spontaneous activity in the nerve cord[29,31]; and it evidently decreases the rate of repolarization of a cockroach nerve which has suffered a long lasting depolarization by exposure to a prolonged cathodal current[32].

In spite of the accumulation of such data, correlations or causal relationships between such changes in nervous function are not clear; furthermore, there are some discrepancies between data. A lowering of the calcium concentration in the surrounding medium acts synergistically on the DDT induced repetitive excitations, while an increasing of the calcium concentration causes the augmentation of the negative after-potential in the vertebrate animals[7,10,14,22-24]. In the insect nerve, however, DDT actually increases the negative after-potential.

Although the increase in both the repetitive excitability and the spontaneous activity under the influence of DDT suggests some close relation between them, no conclusive evidence is available at the present time[31]. The increase in the negative after-potential and the delay of repolarization after a prolonged cathodal current also suggests some close correlation.

Such changes in the recovery process as an increase in the negative after-potential, the delayed repolarization, and the increase in the supernormal phase, may be induced either by a disturbance of the metabolism concerned or by an alteration of the ionic permeability of the nerve membrane. In order to clarify the mechanism of increase in the negative after-potential by DDT, it is necessary to examine the effect of various environmental factors, such as potassium and calcium concentrations, pH, electrotonus, and metabolic inhibitors on the negative after-potential induced by DDT. On the other hand, it is also necessary to find the relationship between the negative after-potential induced by DDT and the augmentation of the repetitive excitability in the nerve under the influence of DDT in order to clarify the mechanism of the unstabilizing action of DDT. Another approach to these problems is by recording the electrotonic potentials of the nerve treated with DDT, because the electrotonic potential closely correlates with the resistance of the nerve membrane. Some of these experiments have already been performed and will be published soon.

The increased negative after-potential induced by DDT is never restored to the normal level even if the poisoned cockroach recovers from the symptoms after a rise in the temperature. This ineffectiveness of the temperature is very surprising, because the unstabilizing action of DDT was found to be much stronger at lower temperatures[30,31].

Summary

The effects of DDT on the negative after-potential and the recovery process of the cockroach nerve were investigated using an amplifer and an oscilloscope.

In the crural nerve of the DDT poisoned cockroach, the negative after-potential was augmented and prolonged markedly in any of the three earlier stages of poisoning symptoms at low temperatures to such a extent that the duration

of the action potential was greater than 2 milliseconds, often over 10 milliseconds. The duration of the action potential in the unpoisoned cockroach was 1.73 millisecond at 16.5°. In the crural nerve of the cockroach which had recovered from the poisoning symptoms after raising the temperature to 29°, the negative after-potential was never decreased.

In the central nerve cord of the poisoned cockroach or in the nerve cord treated with DDT after excision from the untreated cockroach, the negative after-potential was also augmented and prolonged markedly often lasting more than 30 milliseconds.

Although the normal or the untreated nerve never showed a supernormal phase, the nerve excised from the DDT poisoned cockroach showed a markedly increased supernormal phase.

These results were discussed in the light of our preceding papers and the literature concerning the mode of action of DDT and the nature of the after-potential in the nerve.

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